JOURNAL OF VIROLOGY, May 2005, p. 5386–5399 Vol. 79, No. 9
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.9.5386–5399.2005

Covert Human Immunodeficiency Virus Replication in Dendritic Cells and in DC-SIGN-Expressing Cells Promotes Long-Term Transmission to Lymphocytes

Cinzia Nobile,[1] Caroline Petit,[2] Arnaud Moris,[1] Katharina Skrabal,[3] Jean-Pierre Abastado,[4] Fabrizio Mammano,[3] and Olivier Schwartz[1]*

Groupe Virus et Immunité, URA CNRS 1930, Institut Pasteur,[1] Institut Cochin,[2] INSERM U552-Recherche Antivirale, Hôpital Bichat,[3] and IDM Research Laboratories, Institut des Cordeliers,[4] Paris, France

Received 27 September 2004/Accepted 6 December 2004

HIV-1 virions are efficiently captured by monocyte-derived immature dendritic cells (iDCs), as well as by cell lines expressing the lectin DC-SIGN. Viral infectivity can be retained for several days, and even enhanced, before transmission to CD4$^+$ lymphocytes. The role of DC-SIGN in viral retention and enhancement of infection is not fully understood and varies according to the cell line expressing the lectin. We studied here the mechanisms underlying this process. We focused our study on X4-tropic human immunodeficiency virus (HIV) strains, since they were widely believed not to replicate in iDCs. However, we first show that X4 HIV replicates covertly and slowly in iDCs. This is also the case in Raji–DC-SIGN cells, which are classically used to study HIV transmission. We used either single-cycle or replicative HIV and measured viral RT and replication to further demonstrate that transfer of incoming virions from iDCs or DC-SIGN$^+$ cells occurs only on the short-term (i.e., a few hours after viral exposure). There is no long-term storage of original HIV particles in these cells. A few days after viral exposure, replicative viruses, and not single-cycle virions, are transmitted to CD4$^+$ cells. The cell-type-dependent activity of DC-SIGN reflects the ability of HIV to replicate covertly in some cells, and not in others.

Human immunodeficiency virus (HIV) subverts the trafficking properties of dendritic cells (DCs) to reach secondary lymphoid organs and to spread to CD4$^+$ T lymphocytes. Virions are steadily captured by DCs. Conjugates between DCs and T cells are easily formed (46, 50), a process which facilitates transmission of HIV by locally concentrating virus on donor cells and viral receptors on target cells during the formation of an infectious synapse (31, 41). In DCs, a variety of molecules can bind gp120, the viral envelope glycoprotein (19, 27, 55, 56). Among them, DC-SIGN (or CD209), a C-type (Ca-dependent) lectin that selectively recognizes high-mannose oligosaccharides (17), plays a peculiar role during virus transmission. DC-SIGN is expressed on some DC subsets, including those derived from blood monocytes or found in lymphoid tissues and beneath genital surfaces (30). DC-SIGN-expressing cells internalize HIV type 1 (HIV-1) virions into a trypsin-resistant compartment, and viral infectivity can be retained for several days and even enhanced before transmission to T cells (2, 6, 19, 36, 54). In addition to DC-SIGN, the CD4 molecule and other lectins such as the mannose receptor and langerin bind gp120 (56), and they may also play a role in virus capture and transmission.

The mechanisms by which DC-SIGN promotes *trans* infection of target cells are not fully understood. Incoming virions are rapidly degraded in DCs and in DC-SIGN$^+$ cells (43, 57). There is thus an apparent discrepancy between the short half-life of incoming virions (<3 h) and the ability of DCs and DC-SIGN$^+$ cell lines to retain and transmit the infection to T cells, which has been observed up to 6 days after viral exposure (19, 45, 54).

Several mechanisms may account for this discordance. First, defective virions, which form the majority of viral preparations, may be more sensitive to degradation than fully infectious particles. Second, cell-cell transmission of virus through the infectious synapse is an efficient and rapid process that may necessitate only minute amounts of virions (26, 41). DC-SIGN internalizes HIV into a low-pH compartment (36). It has been proposed that virus recycling from this compartment will lead to infectivity enhancement and transmission (36). This intracellular compartment has not yet been identified, and the nature of the pH-dependent process enhancing infectivity is not understood. Interestingly, the properties of DC-SIGN when expressed on immature DCs (iDCs) can be recapitulated in Raji DC-SIGN B cells, which allow viral transfer on the long term, as well as DC-SIGN-mediated *trans* enhancement (19, 36, 54, 58). However, a cell-type-dependent activity of DC-SIGN has been documented, and other cell lines, such as THP1, K562, or 293 cells expressing the lectin, are unable to perform these tasks (54, 58, 59). The origin of this cell-type-dependent effect has not been deciphered.

Third, a nonexclusive possibility is that progeny viruses, rather than input virions, are transmitted from DCs to lymphocytes. HIV-1 replicates rather inefficiently in DC cultures. DCs express low levels of CD4 and coreceptors CCR5 or CXCR4. It has been reported that R5, but not X4, HIV-1 strains replicate in immature monocyte-derived DCs (21, 45, 47). However, both R5 and X4 viruses readily enter DCs and are able to perform reverse transcription (RT) in these cells (3, 9, 14, 22–24). Of note, both X4 and R5 strains are efficiently

* Corresponding author. Mailing address: Institut Pasteur, Groupe Virus et Immunité, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France. Phone: 33 1 45 68 83 53. Fax: 33 1 40 61 34 65. E-mail: schwartz@pasteur.fr.

transmitted, even a few days after viral exposure, from DCs to lymphocytes (19, 45). However, with R5 strains, which replicate in DCs, viral progeny (but not incoming virions) are transmitted after a few hours (57). It is thus conceivable that a previously underestimated, surreptitious replication of X4 isolates in DCs will be the main source of an infection spreading towards the lymphocytes.

In this report, we examined the process of HIV transmission from iDCs or DC-SIGN-expressing cells to target cells. We focused our study on X4 strains, since they were previously believed not to replicate in iDCs. In contrast to these initial reports, we show that X4 strains replicate consistently at low levels in monocyte-derived iDCs. By using either single-cycle or replicative HIV and measuring viral RT and replication, we further demonstrate that a short-term (i.e., a few hours after viral exposure), pH-independent transfer of incoming virions occurs in iDCs or in DC-SIGN-expressing cells. However, there is no long-term storage of infectious HIV particles in these cells. A few days after viral exposure, progeny viruses (rather than incoming virions) are transmitted to lymphocytes. The cell-type-dependent activity of DC-SIGN on long-term virus transmission simply reflects the ability of HIV to replicate covertly in some cells and not in others.

MATERIALS AND METHODS

Cells and reagents. DCs were prepared with a VacCell processor as previously described (20). Briefly, peripheral blood mononuclear cells from leukapheresis were cultured for 7 days in serum-free VacCell medium (Invitrogen) supplemented with 500 U of granulocyte-macrophage colony-stimulating factor (Novartis, France)/ml and 50 ng of interleukin-13 (Sanofi-Synthelabo, France)/ml, and DCs were isolated by elutriation. The DC isolation procedure yielded $CD1a^+$, major histocompatibility complex class I- and class II-, DC-$SIGN^+$, $CD64^-$, $CD83^-$, CD80-low, and CD86-low cells, a phenotype corresponding to iDCs. Preparations routinely contained about 2% $CD3^+$ cells. C1RA2 DC-SIGN cells were derived from C1R-A2 by infection with a lentiviral vector encoding DC-SIGN and sorting DC-SIGN-positive cells (43). Raji and Raji DC-SIGN cells were a kind gift of Ali Amara (19, 58). P4 cells are HeLa $CD4^+$ cells carrying a HIV long terminal repeat (LTR) *lacZ* reporter cassette. HeLa cells were transiently transfected with plasmids encoding CD4 and/or DC-SIGN as previously described (37, 51). Cells were infected with HIV 24 h after transfection. Bafilomycin A1 and concanamycin A were from Sigma, T-20 was from American peptide, and zidovudine (AZT) and nevirapine (NVP) were from the National Institutes of Health AIDS Research and Reference Reagent Program.

Viruses and infections. HIV (NL4.3 and NLAD8 strains) and HIV(vesicular stomatitis virus [VSV]) virions were produced and titrated as previously described (40). NLAD8 was a kind gift of Eric Freed. NL F522Y provirus encodes a nonfusogenic gp120/g41 complex (8, 11). Single-cycle pseudotyped HIV particles [HIV-Luc and HIV-Luc(VSV)] were generated by cotransfection of 293T cells with the proviral vector pNL-Luc E-R- containing the firefly luciferase gene (15) and with an gp120/gp41 expression vector (from the X4 strain HXB2) or the VSV-G envelope glycoproteins, respectively. Proviral vectors encoding for patient-derived envelope glycoproteins were generated by cloning primary *env* sequences, spanning the entire gp120 domain and most of gp41, into a pNL4-3 variant (pNL43XC-MS2), which harbors an MluI site in C1 of *env* (nucleotide 6435) in addition to the natural BamHI site (nucleotide 8465). The primary envelope genes were obtained by RT-PCR amplification from a plasma sample issued from a treatment-naive, symptomatic patient and were cloned in MluI-BamHI. Several replication competent viral clones were obtained and sequenced (K. Skrabal et al., unpublished data). The tropism of the corresponding viruses was determined by infection of U373-MG-CD4-CCR5 and U373-MG-CD4-CXCR4 reporter cells. Infection of iDCs and other cells was described previously (45). iDCs (10^6) were exposed to the indicated viruses for 2 to 3 h at 37°C, extensively washed, and grown for the indicated periods of time. Virus release was monitored by measuring p24 production in supernatants by enzyme-linked immunosorbent assay (ELISA) (Perkin-Elmer Life Sciences). When stated, AZT, NVP, or T-20 was added 1 h before virus exposure and maintained throughout the assay.

Flow cytometry. Cells were stained with anti-DC-SIGN (monoclonal antibody [MAb] 161-PE; R&D), anti-mannose receptor (MR) (MAb 3.29B1.10; Coulter), anti-CD3 (SK7-PE; Becton Dickinson) or isotype MAbs as a negative control; permeabilized; and intracellularly stained with anti-Gagp24-FITC MAb (KC57; Coulter). Cells were analyzed by flow cytometry with a FACSCalibur cytometer (Becton-Dickinson).

HIV capture assay. iDCs were exposed to the indicated viruses (30 or 6 ng of p24/5 per 10^5 cells) for 2 h at 37°C. Cells were then extensively washed and lysed in 0.5% Triton buffer. Lysates were centrifuged (5 min at 10,000 rpm) to discard cell debris before p24 measurement by ELISA.

HIV transmission assay. Donor cells (5×10^5 to 1.5×10^6) were incubated with the indicated doses of HIV for 2 h at 37°C. Cells were then washed thoroughly and 2×10^4 donor cells were added to an equal number of target cells (HeLa, HeLa $CD4^+$, MT4, or Jurkat cells), either immediately or after the indicated periods of time. For experiments performed with single-cycle HIV-Luc, cells were harvested after an additional 48-h culture period. Luciferase activity was measured with a luciferase reporter assay kit (Promega) and a luminometer (Perkin-Elmer). Of note, in some experiments (see Fig. 5 and 8), luciferase activities were measured with another luminometer (Berthold Technologies), which yielded higher luminescence signals. Luciferase levels cannot thus be compared to values depicted in some figures (e.g., see Fig. 4, 6, and 9). For experiments performed with replicative HIV, virus transmission was assessed by measuring β-galactosidase activity in P4 cell extracts after a 2-day coculture period. When stated, bafilomycin A1 or concanamicyn A were added 1 h before and maintained during virus exposure. Cells were then extensively washed to remove drugs and grown in culture medium.

Quantitative PCR. To minimize contamination of HIV stocks with plasmidic or cellular DNA, NL4.3 and NLAD8 viruses were produced by infection of MT4-CCR5 cells and harvesting cell supernatants before the occurrence of virus-induced cytopathic effects. NL F522Y (and wild-type [WT] NL4.3 as a control) virus stocks were prepared by a two-step procedure. First, VSV-G-pseudotyped virions were generated by cotransfection of HeLa cells. Second, the resulting viral supernatants were used to infect Jurkat cells. Supernatants of Jurkat cells were collected a few days after infection and used for further studies. HIV infections were performed with the indicated viruses (at 50 ng of p24/ml/10^6 cells). At various time points, DNA was prepared from infected cells with the Qiaamp DNA extraction kit (Qiagen). Total HIV DNA was measured with primers in the RU5 LTR region, as previously described (10). Sequences of the primers (purchased from TIB MOLBIOL) were as follows: forward primer M667 (5′-GGC TAA CTA GGG AAC CCA CTG-3′) and reverse primer AASM (5′-GCT AGA GAT TTT CCA CAC TGA CTAA-3′). The fluorogenic hybridization probes used to detect the amplification products were LTR-FL (5′-CAC AAC AGA CGG GCA CAC ACT ACT TGA-3′) and LTR-LC (5′-LC Red640-5′-CAC TCA AGG CAA GCT TTA TTG AGG C p-3′). After initial incubation at 95°C for 8 min, 50 cycles of amplification were carried out for 10 s at 95°C, followed by 10 s at 60°C and 8 s at 72°C. Complete RT products were quantified with primers in the *Pol* region (HIV-Pol F forward, 5′-TTT AGA TGG AAT AGA TAA GGC CCA A-3′; HIV-Pol R reverse, 5′-CAG CTG GCT ACT ATT TCT TTT GCT A-3′); the fluorogenic *Taq*Man probe used to detect the amplification products was Pol_TM (5′- 6-carboxyfluorescein-AAT CAC TAG CCA TTG CTC TCC AAT TAC). After initial incubation at 95°C for 8 min, 50 cycles of amplification were carried out, each consisting of 5 s at 95°C, followed by 30 s at 62°C. Reaction mixtures contained 1× LightCycler-FastStart DNA Master Hybridization probes (Roche), 300 nM each primer, 200 nM each probe, and 5 μl of template DNA in a 20-μl volume. For each experiment, a standard curve of the amplicon being measured was run in duplicate ranging from 10 to 10^5 copies, plus a no-template control. The pNL-43 plasmid was used for the standard curve of viral DNAs. Quantitation of the human β-globin gene was performed with LightCycler-Control Kit DNA and was used to determine the number of cells. Reactions were performed with a Light Cycler and analyzed with the manufacturer's software (version 3.5). Data are expressed as the numbers of viral DNA copies per cell.

RESULTS

Low levels of X4 HIV replication in iDCs. To investigate the mechanisms of HIV transmission from DCs to lymphocytes, we first compared the replicative capacities of the R5-tropic NLAD8 and X4-tropic NL4.3 isolates in DCs. It has been reported that R5, and not X4, HIV-1 strains replicate in iDCs (21, 38, 45). However, both R5 and X4 HIV-1 readily bind and

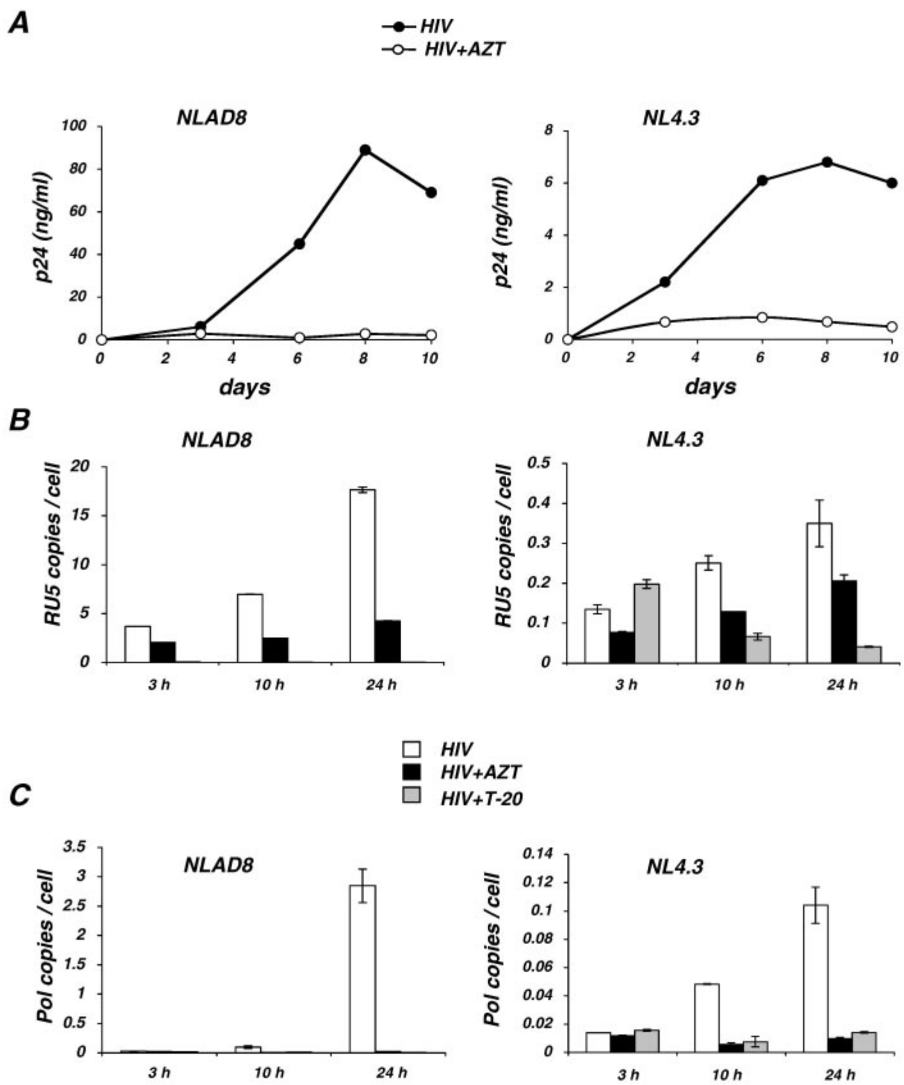

FIG. 1. HIV replication and proviral DNA synthesis in monocyte-derived iDCs. (A) HIV replication. iDCs (10^6 cells) were exposed to the indicated viruses (50 ng of p24), with or without AZT (10 μM). After overnight incubation, cells were washed to remove unbound virus. Viral replication was monitored by measuring p24 production in culture supernatants. One of six independent experiments is shown. (B and C) HIV proviral DNA synthesis. iDCs were exposed to the indicated viruses (50 ng of p24/10^6 cells) for 3 h and extensively washed. Cell aliquots were then immediately collected or incubated at 37°C for various periods of time. Quantification of early (RU5 DNA) (B) and late (Pol DNA) (C) viral products was performed by real-time PCR. Data are means ± standard deviation of triplicates and are representative of at least three independent experiments, performed with DCs from different donors.

enter DCs (3, 13, 22, 35). We thus examined whether this efficient capture may lead to low levels of X4 viral replication that could have been previously underestimated. To this aim, iDCs were derived from primary monocytes. Cells expressed classical surface markers of bona fide iDCs (see Materials and Methods), as well as low surface levels of CD4 and coreceptors, as reported previously (45). To increase the sensitivity of detection of virus replication, iDCs were cultivated at a high concentration (10^6 cells/ml) and exposed to a relatively high viral inoculum (50 ng of p24/10^6 cells). Virus replication was then assessed by measuring p24 production in cell supernatants. As expected, the R5 HIV strain replicated quite efficiently, and p24 production reached 80 ng/ml at day 8 postinfection (p.i.) (Fig. 1A). Interestingly, under these experimental conditions, a low but significant level of viral production was detected with the X4 strain NL4.3, with a peak of 6 ng of p24/ml at day 8 p.i. Using cells from six different donors, we consistently detected viral production reaching 1 to 10 ng of p24/ml (data not shown). This p24 production was not due to a regurgitation of the viral inoculum, since it was inhibited by two reverse transcriptase inhibitors, AZT (Fig. 1A) or NVP (data not shown).

We then quantified the efficiency of RT for R5 and X4 viruses in iDCs by performing a real-time PCR analysis. iDCs were exposed to viral inocula for 2 h (50 ng of p24/10^6 cells), supernatants were removed, and cells were harvested as a function of time after initiating infection. DNA samples were then analyzed for the presence of early (RU5) and late (pol)

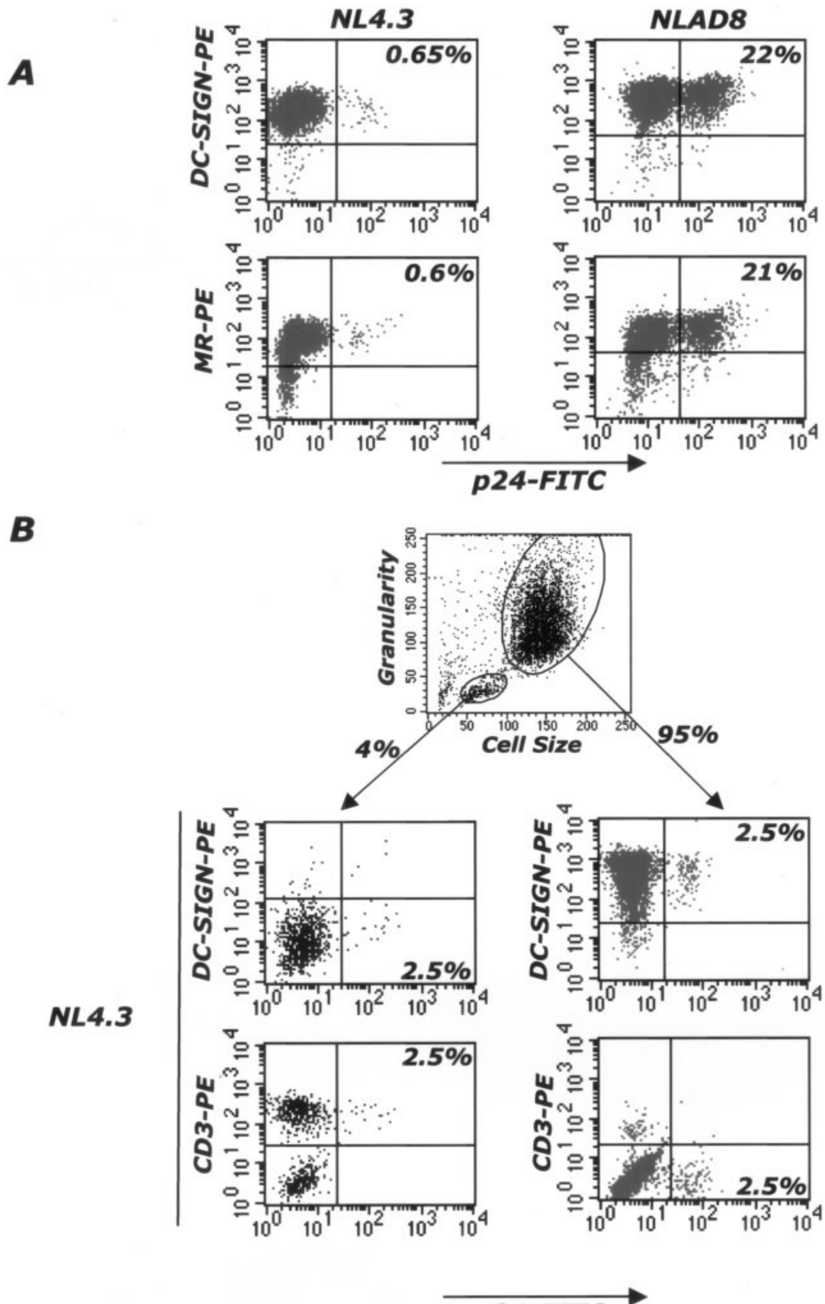

FIG. 2. Intracellular p24 expression by HIV-infected iDCs. (A) DCs from one donor were exposed to the indicated viruses (50 ng of p24/10^6 cells) for 2 h at 37°C. Five days later, fluorescence-activated cell sorter analysis was performed to monitor HIV infection (p24-FITC staining, *x* axis) and the surface proteins DC-SIGN and MR (phycoerythrin staining, *y* axis). (B) iDCs from another donor were exposed to the X4 strain NL4.3 (50 ng of p24/10^6 cells). Five days later, cells were stained for p24, DC-SIGN, MR, or CD3. Similar results were obtained with cells from four different donors.

viral RT products (Fig. 1B). With NLAD8, we observed an increase of total RT products over time, reaching 20 RU5 copies per cell at 24 h after infection. The late viral DNA products were less abundant, became detectable after 10 h of infection, and reached 3 pol copies per cell at 24 h. Viral DNA synthesis was also observed with NL4.3, albeit at a much lower level. After 24 h of infection, RU5 and pol copies were in the range of 0.4 and 0.1 copies per cell, respectively. AZT was included as a control to ensure that the detected PCR products were the result of proviral DNA neosynthesis (Fig. 1B). Altogether, these results indicate that the low levels of NL4.3 replication in iDCS, when compared to NLAD8, are associated with a 20- to 40-fold decrease in the efficiency of the RT process.

We then examined whether iDCs were the major source of virus in infected cultures. Cells were double stained with Gag

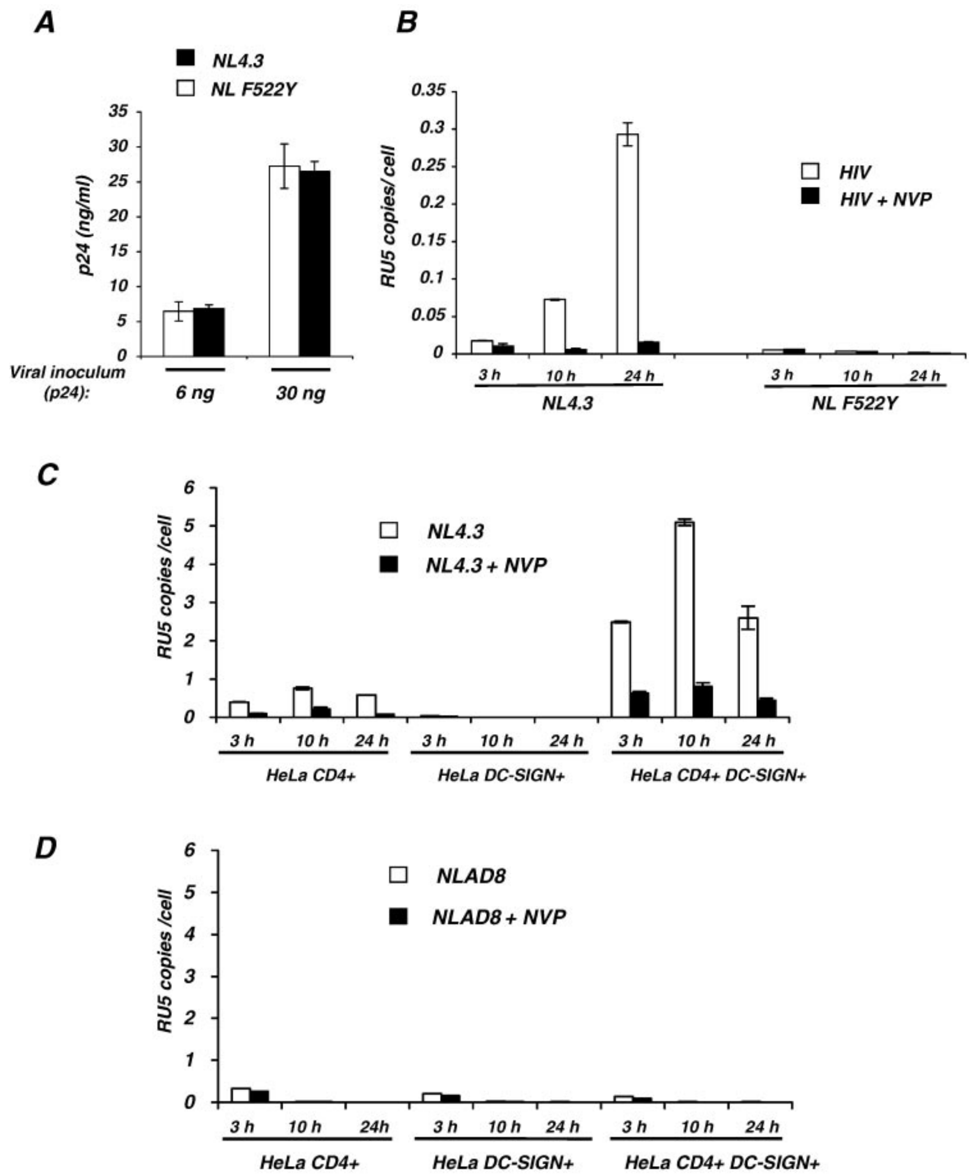

FIG. 3. HIV RT in iDCs requires viral fusion. (A) Capture of WT (NL4.3) and fusion-defective (NL F522Y) HIV by iDCs. Cells were exposed to the indicated viruses (6 or 30 ng of p24/10^6 cells) for 2 h at 37°C and washed extensively, and cell-associated p24 was quantified by ELISA. (B) RT in iDCs. Cells were exposed to the indicated viruses as described in the legend to Fig. 1. Quantification of RU5 viral DNA was performed by real time PCR. (C-D) RT in HeLa cells. Cells were transiently transfected with CD4 and/or DC-SIGN and were exposed to NL4.3 (C) or NLAD8 (D). RU5 viral products were quantified by real-time PCR as described in the legend to Fig. 1. Data are means ± standard deviation of triplicates and are representative of at least three independent experiments.

p24 and cell lineage markers at day 5 p.i. and analyzed by flow cytometry (Fig. 2A). Most of the $p24^+$ cells were positive for the DC markers DC-SIGN and MR. With NLAD8, according to donors, between 10 to 60% of DC-SIGN$^+$ cells expressed Gag antigens. With NL4.3, the fraction of $p24^+$ DC-SIGN$^+$ cells varied between 0.5 to 3.5% with NL4.3 (mean of 2.2 ± 1.2%, with cells from four independent donors). DC preparations routinely contained low levels of lymphocytes (1.8 ± 1.5% of cells are CD3$^+$, mean of four independent DC preparations), which could be gated according to their size and granularity (Fig. 2B). A subset of these low levels of CD3$^+$ lymphocytes was productively infected by HIV (according to donor samples, 2.7 ± 2% of CD3$^+$ cells expressed Gag p24; mean of four experiments). Therefore, in our cell cultures, iDCs represented the major source of Gag-expressing cells when compared to lymphocytes (a mean of 2.2% of total cells were Gag$^+$ DC-SIGN$^+$, and 0.05% were Gag$^+$ CD3$^+$). Moreover, there was no evident correlation between the percentage of lymphocytes in a given culture and the levels of p24 production in supernatants (data not shown). This confirmed that most of the released virions were produced by DCs (among Gag$^+$ cells, 98% expressed DC markers, and 2% expressed T-cell markers). Of note, Gag p24 staining was not caused by the viral inoculum, since it was inhibited by NVP (not shown).

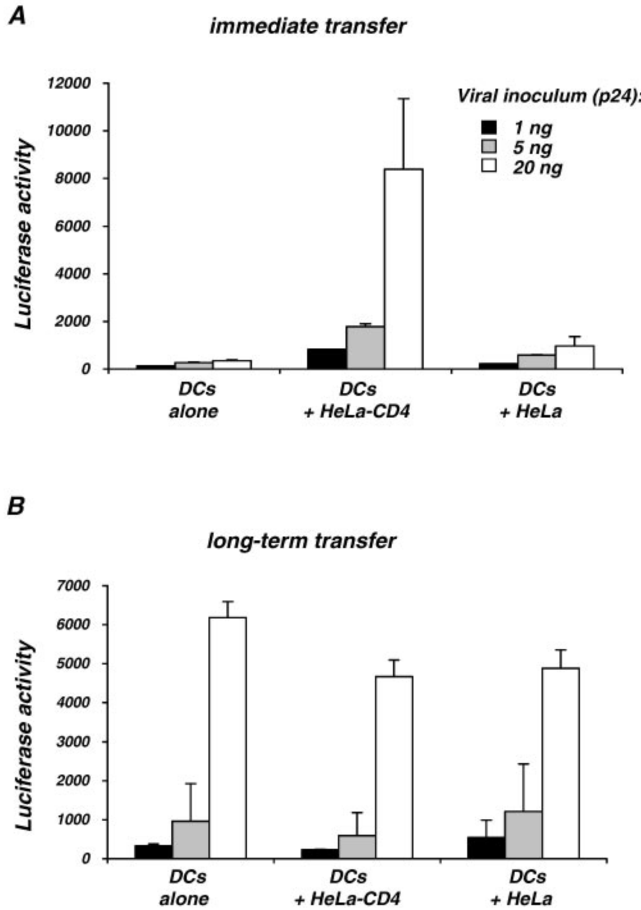

FIG. 4. Capture and transmission of incoming HIV by iDCs. iDCs (10^6 cells) were exposed to the indicated doses of single-cycle HIV-Luc (pseudotyped with X4 envelope glycoproteins) and extensively washed. Cells were then either cultured alone or with target HeLa or HeLa-CD4 cells. Cocultures were performed either immediately (immediate transfer) (A) or 48 h after viral exposure (long-term transfer) (B). Cell lysates were obtained after an additional 48-h culture period and analyzed for luciferase activity (in relative light units). Data represent the means ± standard deviation of three separate wells of infected cells and are representative of at least three independent experiments.

We concluded that the X4 HIV strain NL4.3 productively infects, at low levels, a small fraction of iDCs.

HIV RT in DCs requires viral fusion. Both R5 and X4 HIV virions are easily internalized by iDCs in intracellular vesicles, in large part through binding of viral envelope glycoproteins to DC-SIGN (9, 19, 41, 44). After capture, DC-SIGN retains virions in an infectious state and transmits them to lymphocytes, with an enhancement of infection efficiency (19). We examined whether this enhancement may be linked to the triggering of RT in intracellular vesicles before or even without any access of incoming virions to the cytoplasm. We thus asked whether HIV RT in iDCs or in DC-SIGN$^+$ cell lines requires viral fusion. To this aim, we first studied the activity of the viral fusion inhibitor T-20 (34). T-20 inhibited proviral DNA synthesis of both NL4.3 and NLAD8 strains (Fig. 1B), suggesting that RT does not occur without viral fusion. However, T-20 was not fully active, in particular at the early time points (3 h) of infection with NL4.3. The small amounts of proviral DNA detected at this time may correspond to partial reverse transcripts present within incoming virions (52). To further rule out the possibility that some fusion-independent proviral DNA synthesis events occur after viral capture, we used virions coated with a fusion-defective HIV-1 envelope (8). This mutant envelope (F522Y) retains the ability to bind CD4 (8) and DC-SIGN (results not shown). Accordingly, both WT and mutant NL4.3 F522Y virions were similarly captured by iDCs (Fig. 3A). However, we did not detect any proviral DNA synthesis by real-time PCR in cells exposed to NL4.3 F522Y (Fig. 3B).

We then documented the role of DC-SIGN during RT. We previously reported that in HeLa cells, DC-SIGN increases the capture and subsequent internalization of incoming virions by about 10 fold (44). We thus used HeLa cells expressing CD4, DC-SIGN, or both molecules as targets and followed RT upon exposure to NL4.3. In HeLa CD4$^+$ cells, the number of total viral DNA copies showed a transient increase, peaking at 10 h after infection, and then declining by 24 h (Fig. 3C). This decline has been reported previously (12) and is likely due to the high cytopathic effect of infection in these cells. In HeLa CD4$^+$ DC-SIGN$^+$ cells, a similar kinetic of RT was observed, with an overall 10-fold increase in the number of RU5 copies per cell. In the absence of CD4, DC-SIGN did not promote RT (Fig. 3C). Viral DNA corresponded to de novo synthesis, since the signal was dramatically decreased in the presence of NVP (Fig. 3C). Of note, HeLa cells lack CCR5, and the R5 isolate NLAD8 was unable to reverse transcribe, even in HeLa DC-SIGN$^+$ CD4$^+$ cells (Fig. 3D).

Altogether, these results indicate that even though HIV virions are efficiently captured and internalized in intracellular vesicles in iDCs and in DC-SIGN$^+$ cell lines, RT does not occur in the absence of a fusion event, which allows access of incoming virions to the cytoplasm of target cells.

Absence of long-term storage of incoming infectious HIV in iDCs. It is widely accepted that iDCs as well as some DC-SIGN$^+$ cell lines retain competence to infect target T cells up to 6 days after viral exposure (19, 36, 45, 54). Our findings that the life span of incoming virions in iDCs is short (2 to 4 h) (43), combined with our observation that X4 strains may replicate covertly in iDCs (Fig. 1 to 3), raise the possibility that progeny virus, rather than incoming virions, is transmitted to lymphocytes after prolonged periods of time. To address this question, we exposed iDCs to various doses of single-cycle HIV-luciferase pseudotyped with X4 envelope glycoproteins (HIV-Luc) (1 to 20 ng of p24/10^6 cells). After 2 h at 37°C, cells were extensively washed, and virus transfer was visualized by cocultivation of iDCs with target HeLa cells, expressing CD4 or not (Fig. 4A). At 48 h later, luciferase activity in cocultures was measured. A positive signal, increasing with the virus inoculum, was detected in the presence of HeLa-CD4 cells, whereas no signal was detected in the absence of target cells or after iDCs were mixed with HeLa cells. These results confirmed that iDCS are able to transfer incoming virions to target cells, if the latter express appropriate viral receptors. We then analyzed the ability of iDCs to retain infectious virions in the long term. To this aim, target cells were added 48 h after iDCs were exposed to the single-cycle reporter virus. Luciferase activity was then measured after an additional period of 48 h (Fig. 4B). With this time frame of 96 h, iDCs became infected and pro-

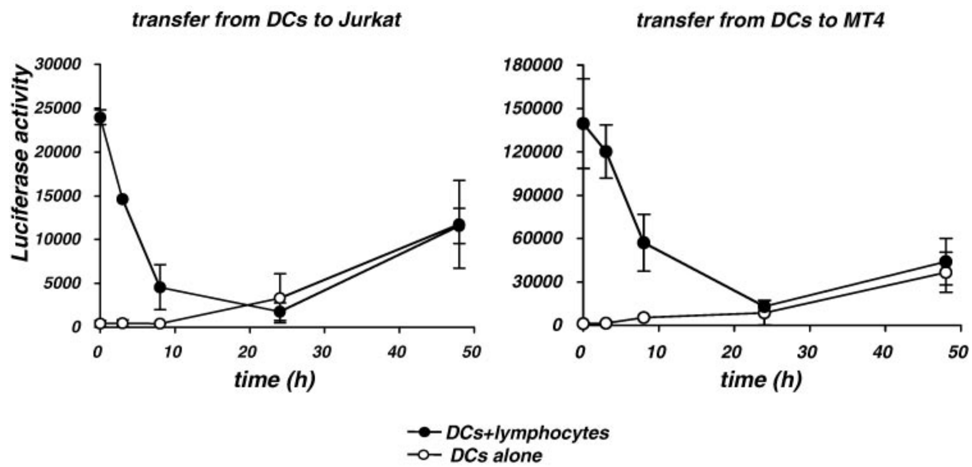

FIG. 5. Transmission of incoming HIV from iDCs to T cells. iDCs were exposed to single-cycle HIV-Luc (pseudotyped with X4 envelope glycoproteins) (100 ng of p24/1.5 $\times$ 10^6 cells) and extensively washed. Cells were then either cultured alone or with target Jurkat (left) or MT4 (right) T cells. Cocultures were initiated at the indicated time points. Cell lysates were obtained after an additional 48-h culture period and analyzed for luciferase activity (in relative light units). Data represent means $\pm$ standard deviation of three separate wells of infected cells and are representative of three independent experiments.

duced luciferase, confirming that iDCs may be infected at low levels by X4-tropic HIV. Interestingly, a signal of similar intensity was detected in iDCs alone and in iDCs mixed with HeLa-CD4 or with control HeLa cells. This strongly suggests that only iDCs and not HeLa-CD4 cells have been infected by HIV-Luc virions. Incoming infectious viral particles have not been stored in iDCs during 48 h.

We then performed a time course experiment to determine how long transmission in the absence of viral replication is able to take place. We used as targets two T-cell lines, MT4 and Jurkat cells, to study virus transfer from iDCs to lymphocytes. iDCs were exposed to HIV-Luc, and target lymphocytes were added at various time points (from 0 to 48 h). Luciferase activity was then measured 48 h later (Fig. 5). At time zero, HIV-Luc was efficiently transmitted from iDCs to either Jurkat or MT4 target cells. However, a rapid decline in luciferase activity was observed. The ability of iDCs to transfer the original virus was reduced by half at approximately 4 to 8 h, and no virus was transferred 24 h after exposure to DCs (Fig. 5). At a later time point (48 h), luciferase activity in iDCs rose, and similar levels were detected with or without T cells, confirming that iDCs became infected at low levels, but no longer transmitted the original virus inoculum to T cells.

Absence of long-term storage of incoming infectious HIV in two DC-SIGN$^+$ B-cell lines. We further documented the role of DC-SIGN in virus transmission. A cell-type dependent activity of DC-SIGN has been reported (54, 58). Some cell lines, such as Raji DC-SIGN or primary DCs, retain viral infectivity for several days, whereas others, such as THP1 DC-SIGN, K562 DC-SIGN, or 293 DC-SIGN, are unable to perform these tasks. We examined whether this cell-type-dependent effect may be linked to the ability of HIV to replicate covertly in some cell lines and not in others. We used two B-cell lines expressing the lectin, Raji DC-SIGN and C1RA2 DC-SIGN (43). Cells were exposed to HIV-luciferase, and we studied their ability to transfer infection immediately or over the long term (after 48 h). Both Raji DC-SIGN and C1RA2 DC-SIGN cells allowed immediate transfer of infection to target HeLa-CD4 and not to HeLa cells (Fig. 6A). Over the long term, the situation between these cell lines was quite different. A luciferase signal was detected with Raji DC-SIGN cells in both the absence and the presence of target cells. This situation is reminiscent of that observed with primary iDCs. HIV-luciferase productively infected Raji DC-SIGN at low levels, and no transmission was detectable 48 h after viral exposure. In contrast, C1RA2 DC-SIGN cells were not detectably infected with HIV-luciferase, and the defective virus was not transmitted after the 48-h incubation period. Of note, similar results were obtained when Jurkat or MT4 cells were used as targets, instead of HeLa CD4 cells (not shown), confirming that Raji DC-SIGN and C1RA2 DC-SIGN cells transfer HIV over the short term only.

We repeated this experiment by using replicative HIV instead of single-cycle virions. We reasoned that if Raji DC-SIGN cells allow low levels of HIV replication, a long-term transmission of replicative virus would then be detected with these cells, whereas this should not be the case for C1RA2 DC-SIGN cells. To unambiguously detect virus transfer and subsequent productive infection of target cells, we used HeLa-CD4 LTR-LacZ cells (P4 cells) as targets and measured activation of the *lacZ* reporter gene (Fig. 6B). Both Raji DC-SIGN and C1RA2 DC-SIGN cells transmitted infection to P4 cells immediately after virus exposure, confirming results obtained with single-cycle virus. Over the long term, virus transfer was only detected with Raji DC-SIGN donor cells. Therefore, the ability of DC-SIGN-expressing cells to retain and transfer viral infection a few days after contact with contact correlates with covert virus replication in these cells.

To confirm that virus replication was actually occurring in Raji DC-SIGN cells, we measured viral DNA synthesis by real-time PCR (Fig. 7). RU5 viral DNA was detected by 3 h p.i. The levels of total HIV DNA increased over 24 h, reaching 0.08 copies per cell. This signal was abrogated in the presence of NVP and was not detected in Raji cells lacking DC-SIGN

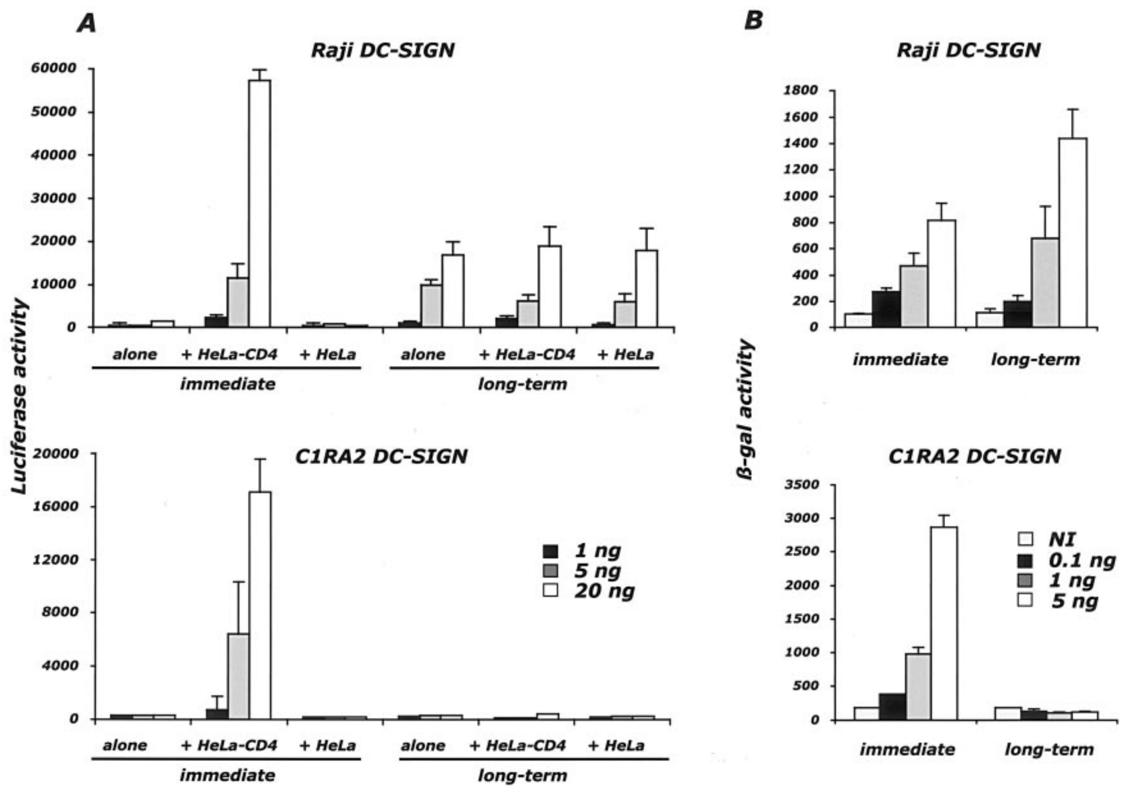

FIG. 6. Capture and transmission of incoming HIV by DC-SIGN-expressing B cell lines. (A) Transmission of single-cycle HIV-Luc. Raji DC-SIGN (top) and C1RA2 DC-SIGN (bottom) cells were exposed to the indicated doses of HIV-Luc. Cells were then either cultured alone or with target HeLa or HeLa-CD4 cells. Cocultures were performed either immediately (immediate transfer) or 48 h after viral exposure (long-term transfer). Cell lysates were obtained after an additional 48-h culture period and analyzed for luciferase activity. Data represent means ± standard deviation of triplicates and are representative of at least three independent experiments. (B) Transmission of replicative HIV. Raji DC-SIGN (top) and C1RA2 DC-SIGN (bottom) were exposed to the indicated doses of NL4.3, washed, and cocultivated with P4 cells (HeLa-CD4 LTR-LacZ reporter cells) either immediately or 48 h after viral exposure. HIV transmission to P4 cells was assessed by measuring β-galactosidase activity in cell extracts after a 2-day coculture. Data (measured by optical density) are means ± standard deviation of triplicates and are representative of three independent experiments.

(Fig. 7). Thus, provirus DNA synthesis occurs in Raji DC-SIGN cells at very low levels. At 24 h p.i., about 60- and 5-fold-less viral DNA was synthesized than in HeLa CD4$^+$ DC-SIGN$^+$ or to primary iDCs, respectively (compare Fig. 3, 4, and 7). Of note, very low levels, if any, of CD4 and CCR5 receptors were detected by flow cytometry at the surface of Raji cells, whereas CXCR4 was correctly expressed (results not shown). We speculate that in the presence of DC-SIGN, low levels of HIV receptors present at the cell surface or in intracellular vesicles will allow fusion of incoming virions, delivery of capsids to the cytosol, and RT.

Viral transfer from DC-SIGN$^+$ cell lines or from iDCs is pH independent. It has been reported that DC-SIGN mediates rapid HIV internalization into a low-pH nonlysosomal compartment, which allows retention and enhancement of infectivity (19, 36). Moreover, neutralization of intravesicular pH by concanamycin A, an inhibitor of vacuolar proton pump, abolished the ability of Raji DC-SIGN cells to enhance virus infection (36), suggesting that DC-SIGN-mediated *trans* enhancement of infection is a pH-dependent process. We reexamined this hypothesis and asked whether viral transfer from DC-SIGN-expressing cells or from iDCs is dependent or independent of the intravesicular pH.

We first used Raji DC-SIGN cells as donor cells and P4 indicator cells as targets and studied the effect of concanamycin A and of bafilomycin A1 on viral transfer. The latter compound is another widely used proton pump inhibitor (1, 16, 40, 57). Both compounds are potent inhibitors of the virus entry that requires acidification for fusion, like VSV. Both drugs were rather toxic for Raji DC-SIGN cells when incubated for long periods of time (data not shown). To reduce toxicity, Raji DC-SIGN cells were pulse treated with either concanamycin A (10 nM) or bafilomycin A1 (250 nM) for 1 h and for an additional 2 h in the presence of single-cycle viruses. Cells were then extensively washed to remove the inhibitors and incubated either without or with target P4 cells for 48 h, and luciferase activity in cell lysates was measured. We checked that both compounds were effective in Raji DC-SIGN cells by using HIV-luciferase virions pseudotyped with VSV-G envelope glycoproteins. As expected, in the absence of P4 target cells, direct infection of Raji DC-SIGN cells by a VSV-G-pseudotyped HIV [HIV-Luc(VSV)] was abolished by bafilomycin A1 or by concanamycin A (Fig. 8A). When a single-cycle virus pseudotyped with HIV envelope glycoproteins was used, we observed a moderate decrease in infection of Raji DC-SIGN (Fig. 8B) that we attributed to moderate toxic side

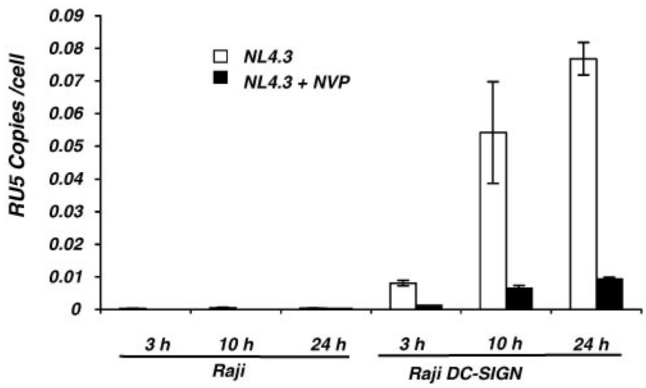

FIG. 7. HIV proviral DNA synthesis in Raji DC-SIGN cells. Raji and Raji DC-SIGN cells were exposed to NL4.3 (50 ng of p24/10^6 cells) as described in the legend to Fig. 1. Quantification of RU5 viral DNA was performed by real-time PCR. Data are means ± standard deviation of triplicates and are representative of three independent experiments.

effects of the inhibitors under these experimental conditions (7). In the presence of P4 cells, the overall luciferase signal was increased, confirming an immediate transfer of virions to these cells. However, this transfer was not affected by concanamycin A or by bafilomycin A1. This indicates that virus transmission from Raji DC-SIGN cells is not a pH-dependent process.

It has been previously reported that at low multiplicities of infection, activated T lymphocytes cocultivated with DCs are more readily infected by HIV than lymphocytes directly exposed to incoming virions (19, 36). We next examined the pH dependency of the transmission of wild-type HIV from iDCs to T lymphocytes (Jurkat cells). In our hands, concanamycin A was rather toxic in iDCs even after extensive washing (data not shown). We therefore employed only bafilomycin A1. When iDCs were treated with bafilomycin A1 for 2 h and then extensively washed to remove the drug, no obvious toxicity was observed during the following 24 to 48 h (not shown). The compound was active in this setting, since infection with HIV-Luc(VSV) pseudotypes was inhibited (Fig. 9A). We then used low viral inputs (0.1 and 0.01 ng of p24/ml/10^6 DCs) to study the effects of bafilomycin A1 on HIV (NL4.3 strain) transmission from DCs to lymphocytes (Jurkat cells). At these multiplicities of infection, very low levels of virus replication, if any, was observed in Jurkat cells alone (Fig. 9B). However, HIV readily replicated after being in contact with DCs. Interestingly, bafilomycin A1 did not affect virus growth in this system. Thus, *trans* enhancement of HIV infection by DCs does not require an acidic pH.

Altogether, these results indicate that virus transfer from DC-SIGN$^+$ cell lines and viral *trans* enhancement from iDCs, are pH-independent phenomena.

Various X4-tropic Env promote HIV replication in iDCs. It was important to verify that the covert viral replication detected in iDCs was not due to special features of NL4.3 Env glycoproteins. We examined whether productive infection of iDCs could be achieved using viruses that carry patient-derived X4 envelopes. To this end, we cloned primary *env* genes, spanning the entire gp120 domain and most of gp41, into an *env*-deleted NL4.3 derivative. The primary envelope sequences were obtained by RT-PCR amplification from a plasma sample issued from an HIV-infected patient. We designed several replication competent viral clones, carrying envelope sequences issued from different variants that coexisted in the plasma viral population (see Materials and Methods for further details). We determined the tropism of these viral clones by infection of U373-MG-CD4-CCR5 and U373-MG-CD4-CXCR4 reporter cell lines (data not shown) (53). For further studies, we selected two X4 (termed T28-X4-1 and T28-X4-2, respectively) and one R5 (T28-R5-1) viral clones (Skrabal et al., unpublished). iDCs (10^6 cells/ml), were exposed to two viral doses (50 and 5 ng of p24/10^6 cells, respectively) for 2 h at 37°C, and viral replication was then assessed by measuring p24 production in cell supernatants. The R5 strain replicated in iDCs, and p24 production reached 30 ng/ml at day 8 p.i. with the larger amount of viral inoculum (Fig. 10). Interestingly, both X4 strains replicated in iDCs, with variable efficiencies. With T28-X4-1, viral production was detected at both high and low inoculum levels, and reached 16 ng of p24/ml at day 10 p.i. Replication of T28-X4-2 was much lower, peaking at 1.4 ng at day 8 pi (Fig. 9). As for NL4.3, we detected Gag p24-expressing DCs by fluorescence-activated cell sorter analysis (data not shown). We conclude that a variety of X4 viruses, carrying laboratory-adapted or primary envelope glycoproteins, productively infect monocyte-derived iDCs at low levels.

DISCUSSION

We show here that after uptake by DCs, HIV virions can be directly transferred to lymphocytes but only during a short period of time (i.e., a few hours). There is no long-term storage of captured virions by DCs or by DC-SIGN-expressing cell lines. After a few days, virus progeny is transmitted to lymphocytes.

We have examined the cellular and virological mechanisms underlying these phenomena. We focused a large part of our study on the behavior of X4 strains, which were widely believed not being able to replicate in iDCs (14, 21, 38, 45, 60). For both X4 and R5 isolates, the maturation state of DCs apparently regulates viral replication. It has been reported that mature DCs (mDCs) display a decreased capacity for the production of HIV, which may be due to a postentry block (4, 21) and/or to variations in CXCR4 and CCR5 expression (14, 60). Whatever the maturation state of these cells, X4 and R5 isolates readily enter DCs, perform RT, and are transmitted to lymphocytes (3, 9, 14, 22, 24, 50). It was thus puzzling that productive infection was not detected with X4 strains in iDCs. We provide here three lines of evidence that X4 strains replicate, albeit at covert levels, in iDCs. First, small amounts of viral production were measured in supernatants of cells exposed to the laboratory-adapted NL4.3 strain, as well as two isolates carrying primary envelope glycoproteins. Flow cytometry analysis confirmed that DCs were productively infected. Viral production at the peak was about 10-fold lower with NL4.3 than in the isogenic R5 strain NLAD8, which differs only in the envelope gene. Second, by using a single-cycle X4 virus expressing the luciferase reporter gene, we detected low levels of luciferase expression in these cells. The viral cycle was apparently slow, since the signal was significantly detectable at 96 h and not at 48 h p.i. Third, quantitative PCR analysis demonstrated

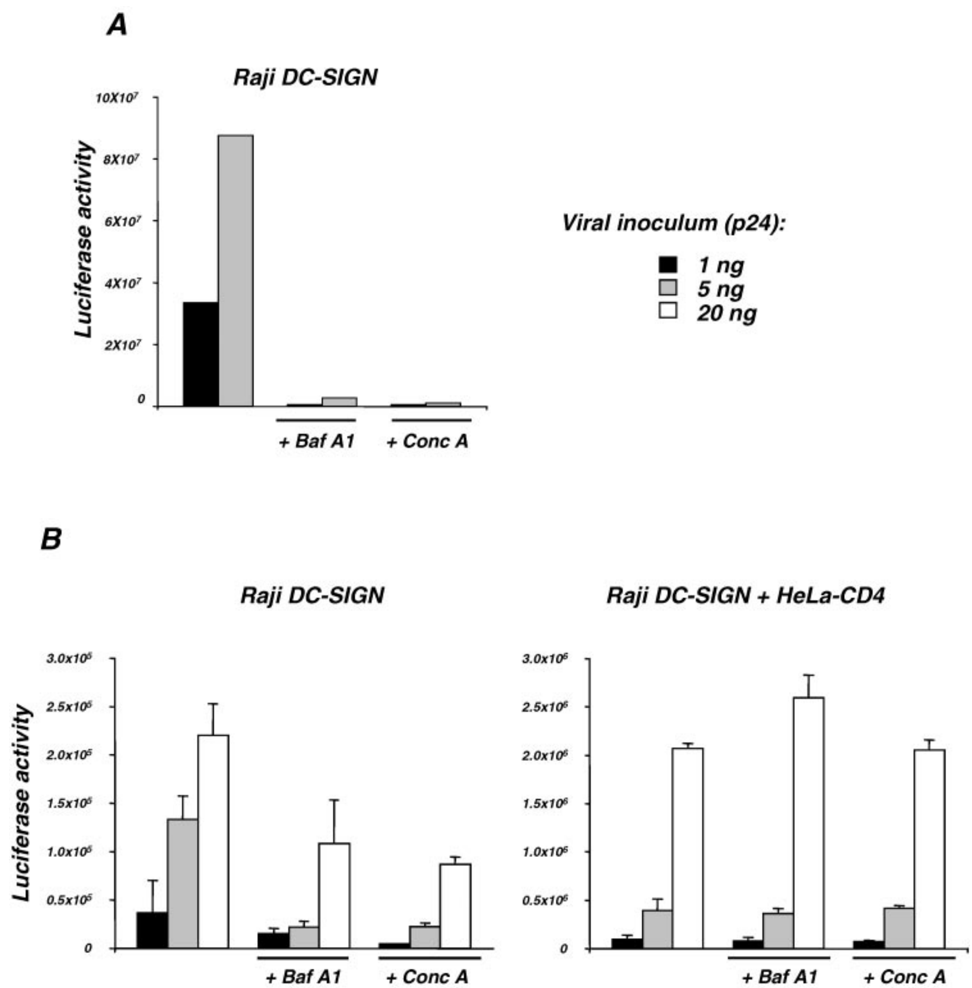

FIG. 8. pH-independent HIV transmission by Raji DC-SIGN cells. (A) Infection by HIV(VSV) pseudotypes is inhibited by bafilomycin A1 and concanamycin A. Raji DC-SIGN cells were pretreated or not pretreated with bafilomycin A1 (Baf A1, 250 nM) or with concanamycin A (Conc A, 10 nM) for 1 h and then pulsed with the indicated doses of HIV-Luc pseudotyped with VSV-G [HIV-Luc(VSV)] for 2 h with or without the drugs. Luciferase activity in cell lysates was measured 2 days later. (B) Effect of bafilomycin A1 and concanamycin A on HIV infection and transmission by Raji DC-SIGN cells. Raji DC-SIGN cells were pretreated or not pretreated with bafilomycin A1 or concanamycin A and then pulsed with the indicated doses of HIV-Luc as described for panel A). Cells were then grown alone (left) or cocultivated with target HeLa-CD4 cells (right). Luciferase activity was measured 2 days later in cell lysates. One out of three independent experiments is shown.

that NL4.3 performed RT in iDCs. Both early and late viral DNA products were synthesized at 20 to 40-fold-lower levels than with NLAD8. Therefore, X4 HIV strains replicate covertly in monocyte-derived iDCs, and this is the consequence of an inefficient early event of the viral cycle occurring at the entry or postentry step.

In iDCs, as well as in DC-SIGN-expressing cell lines, a large portion of incoming virions is internalized in intracellular vesicles (9, 41, 42, 44). We show here that in DCs, proviral DNA synthesis is blocked by T-20, a viral fusion inhibitor, and does not occur with a mutant HIV carrying nonfusogenic viral envelope glycoproteins. Furthermore, by using HeLa cells expressing either DC-SIGN, CD4, or both molecules, we demonstrate that DC-SIGN by itself does not allow RT but significantly enhances viral DNA synthesis in cells expressing appropriate viral receptors (CD4 and CXCR4). Altogether, these results indicate that efficient proviral DNA synthesis requires access of incoming virions to the cytosol. After capture by DC-SIGN and internalization in a vesicular compartment, the so-called natural endogenous RT process (61) does not occur and is therefore not involved in the *trans* enhancement of viral infectivity from DCs to lymphocytes.

In what form is viral infectivity is transmitted from DCs and what is the role of DC-SIGN in this process? By using single-cycle HIV-Luc virions, we show that incoming particles are transferred from iDCs, as well as from Raji DC-SIGN and C1RA2 DC-SIGN cells, to target cells immediately after viral exposure. Parental Raji and C1RA2 cells did not transfer infectivity, confirming the role of the lectin in this process. However, no increase of luciferase signal was detected if target cells were added 48 h after viral exposure, indicating that DC-SIGN-expressing cells do not protect virus inoculum in the long term. Interestingly, in Raji DC-SIGN cells, a significant luciferase activity was detected even in the absence of targets, a situation reminiscent of primary iDCs. Low-level proviral DNA synthesis was detected in Raji DC-SIGN cells (with a

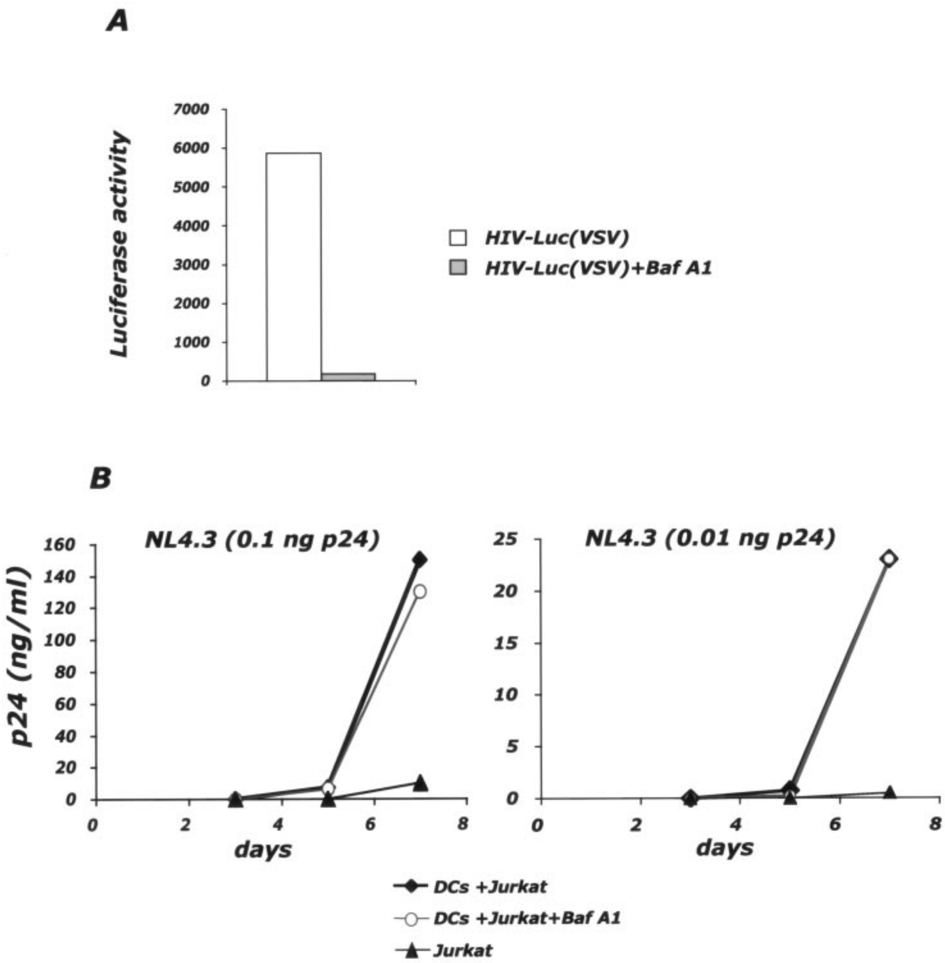

FIG. 9. pH-independent HIV transmission by iDCs. (A) Infection of iDCs by HIV(VSV) pseudotypes is inhibited by bafilomycin A1. iDCs were pretreated or not pretreated with bafilomycin A1 (Baf A1, 250 nM) for 1 h, and then pulsed with HIV-Luc(VSV) (20 ng of p24/10^6 cells). Luciferase activity was measured 2 days later in cell lysates. (B) Bafilomycin A1 does not affect HIV transmission from iDCs to Jurkat cells. iDCs (5×10^5 cells) were pretreated or not pretreated for 1 h with bafilomycin A1 or mock treated, exposed to 0.1 (left) or 0.01 (right) ng of NL4./ml for 2 h, washed, and cocultivated with Jurkat cells (5×10^5 cells). As a control, Jurkat cells were exposed to the same virus inputs and cultured alone. Viral replication was monitored by measuring p24 production in culture supernatants. One of three independent experiments is shown.

60-fold-lower efficiency than HeLa CD4$^+$ DC-SIGN$^+$ cells), confirming the occurrence of surreptitious HIV replication in these cells. Interestingly, replicative HIV and not single-cycle virus was transmitted from Raji DC-SIGN cells 48 h after virus exposure. Altogether, these results indicate that in the long term, only progeny virus is transmitted from Raji DC-SIGN cells or from iDCs to lymphocytes. Turville et al. recently reported that DCs transfer R5 HIV to CD4$^+$ lymphocytes in two distinct phases. By using replicative R5 HIV, they showed that transfer of infectious virus shortly after uptake does not require de novo synthesis, whereas the second phase of transfer is inhibited by AZT and is dependent on productive infection of iDCs (57). Our experiments confirm and extend these observations. We demonstrate here that the transfer of X4 strains follows the same rules, with an immediate phase of transmission of incoming virions and a second phase of delivery of neosynthesized virus. Moreover, we point out that the role of DC-SIGN is mainly at the phase of virus uptake. Most of the incoming virions are rapidly degraded (within hours), whereas only a small fraction reaches the contact zone when DCs interact with T cells. DC-SIGN does not protect incoming virions over the long term, at least in iDCs and the cell lines studied here. The situation may be different in mDCs, which efficiently transmit HIV in the absence of detectable productive infection (21). Upon DC maturation, DC-SIGN is downregulated, the endocytic capacity of the cell is decreased, and HIV virions accumulate in vacuoles which differ in size and intracellular localization from iDCs (18). The half-lives of incoming virions are also short in mDCs, but the rate of viral decay is slightly slower and less extensive than in iDCs (57). It is thus conceivable that mDCs retain the ability to transmit captured virions for longer periods of time. On the other hand, it will be worth reexamining whether productive infection occurs at particularly low levels in these cells.

Our results provide a simple explanation for the cell-type-dependent effect of DC-SIGN on long-term retention of viral infectivity (54). We show here that Raji DC-SIGN cells share with iDCs the capacity to replicate HIV at low levels and

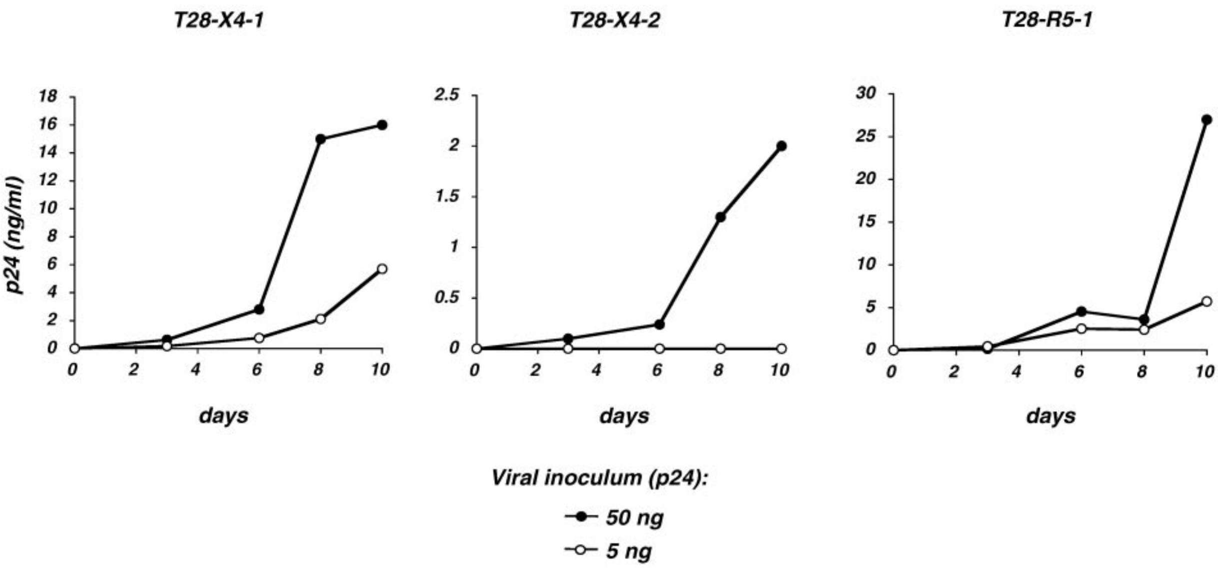

FIG. 10. Replication of HIV strains carrying primary X4 and R5 envelope glycoproteins in iDCs. The primary envelope genes were obtained by RT-PCR amplification from a plasma sample issued from an HIV-infected patient and cloned in an *env*-deleted NL4.3 HIV isolate. Two X4 (T28-X4-1 and T28-X4-2) and one R5 (T28-R5-1) isolates were analyzed. iDCs were exposed to the indicated viruses (50 or 5 ng of p24/10^6 cells). After overnight incubation, cells were washed to remove unbound virus. Virus replication was monitored by measuring p24 production in culture supernatants. One of three independent experiments is shown.

thereby to transfer infection to virus inoculum a few days after the initial exposure. In other cell lines, such as HeLa DC-SIGN, 293 DC-SIGN, or C1RA2 DC-SIGN, the absence of productive infection precludes any retention of viral infectivity.

DC-SIGN also promotes the so-called *trans* enhancement of HIV infection, through a poorly characterized mechanism. Small amounts of virus, insufficient to allow the direct infection of T cells, become infectious after transiting by DCs or DC-SIGN-expressing cells (19, 36). DCs enhance infection through the formation of an infectious synapse, which brings virus and receptors closer together at the contact zone (41). Formation of this synapse is important for virions in transit, but also for transfer of newly synthesized virus particles (29) (31). On the other hand, it has been proposed that DC-SIGN-mediated internalization of incoming HIV is required for *trans* enhancement (36). In this last report, *trans* enhancement was blocked by concanamycin A, an inhibitor of vesicular acidification (36). In contrast, we show here that neither concanamycin A nor bafilomycin A1 inhibited transfer of replicative virus from iDCs to lymphocytes or transmission of single-cycle virus from Raji DC-SIGN to HeLa-CD4 cells. Of note, we used experimental conditions (low doses, pulse incubation, and extensive washing) to minimize the toxicity of these compounds. These drugs are known to affect cell viability, and various side effects have been reported that may bias the interpretation of experiments aimed at raising acidic pH (7). We conclude that virus transfer from iDCs or from DC-SIGN-expressing cell lines does not require the low-pH environment encountered in vesicular compartments. DC-SIGN-mediated enhancement has been previously observed with single-cycle virus and thus involves transfer of incoming virions (19, 36). However, our results indicate that in donor cells where virus replicates at low levels, such as iDCs or Raji DC-SIGN cells, a large part of the *trans* enhancement process is due to the dissemination of freshly produced virus.

R5 strains are preferentially transmitted among humans (39, 62). This restriction process is likely multifactorial and has been suggested to take place at the stage of DC infection (49). We show here that X4 strains replicate in iDCs, albeit at much lower levels than R5 viruses, and are then efficiently transmitted to lymphocytes. Low levels of productive X4 HIV replication have also been observed in Langerhans cell-like DCs (33, 63), as well as in more complex models of HIV dissemination, such as ex vivo culture explants of cervical tissue (25, 28). This should be taken into account when designing strategies aimed at blocking HIV-1 uptake by DCs or other cells within genital mucosa (5, 32, 48).

ACKNOWLEDGMENTS

We thank Nathalie Sol-Foulon and Frédéric Delebecque for critical reading of the manuscript, Françoise Porrot and Patricia Metais-Cunha for excellent technical help, and Eric Freed, Ali Amara, and the NIH AIDS Research and Reference Reagent Program for the kind gift of reagents.

This work was supported by grants from the Agence Nationale de Recherche sur le SIDA (ANRS), SIDACTION, the European Community (grant QLK2-CT 2000-01630), and Institut Pasteur. C.N. is a fellow of ANRS.

REFERENCES

1. **Aiken, C.** 1997. Pseudotyping human immunodeficiency virus type 1 (HIV-1) by the glycoprotein of VSV targets HIV-1 entry to an endocytic pathway and suppresses both the requirement for Nef and the sensitivity to cyclosporin A. J. Virol. **71:**5871–5877.
2. **Arrighi, J. F., M. Pion, M. Wiznerowicz, T. B. Geijtenbeek, E. Garcia, S. Abraham, F. Leuba, V. Dutoit, O. Ducrey-Rundquist, Y. van Kooyk, D. Trono, and V. Piguet.** 2004. Lentivirus-mediated RNA interference of DC-SIGN expression inhibits human immunodeficiency virus transmission from dendritic cells to T cells. J. Virol. **78:**10848–10855.
3. **Ayehunie, S., E. A. Garcia-Zepeda, J. A. Hoxie, R. Horuk, T. S. Kupper, A. D.**

Luster, and R. M. Ruprecht. 1997. Human immunodeficiency virus-1 entry into purified blood dendritic cells through CC and CXC chemokine coreceptors. Blood **90:**1379–1386.
4. **Bakri, Y., C. Schiffer, V. Zennou, P. Charneau, E. Kahn, A. Benjouad, J. C. Gluckman, and B. Canque.** 2001. The maturation of dendritic cells results in postintegration inhibition of HIV-1 replication. J. Immunol. **166:**3780–3788.
5. **Baribaud, F., R. W. Doms, and S. Pohlmann.** 2002. The role of DC-SIGN and DC-SIGNR in HIV and Ebola virus infection: can potential therapeutics block virus transmission and dissemination? Expert Opin. Ther. Targets **6:**423–431.
6. **Baribaud, F., S. Pohlmann, and R. W. Doms.** 2001. The role of DC-SIGN and DC-SIGNR in HIV and SIV attachment, infection, and transmission. Virology **286:**1–6.
7. **Bayer, N., D. Schober, E. Prchla, R. F. Murphy, D. Blaas, and R. Fuchs.** 1998. Effect of bafilomycin A1 and nocodazole on endocytic transport in HeLa cells: implications for viral uncoating and infection. J. Virol. **72:**9645–9655.
8. **Bergeron, L., N. Sullivan, and J. Sodroski.** 1992. Target cell-specific determinants of membrane fusion within the human immunodeficiency virus type 1 gp120 third variable region and gp41 amino terminus. J. Virol. **66:**2389–2397.
9. **Blauvelt, A., H. Asada, M. W. Saville, V. Klaus Kovtun, D. J. Altman, R. Yarchoan, and S. I. Katz.** 1997. Productive infection of dendritic cells by HIV-1 and their ability to capture virus are mediated through separate pathways. J. Clin. Investig. **100:**2043–2053.
10. **Brussel, A., and P. Sonigo.** 2003. Analysis of early human immunodeficiency virus type 1 DNA synthesis by use of a new sensitive assay for quantifying integrated provirus. J. Virol. **77:**10119–10124.
11. **Buseyne, F., S. Le Gall, C. Boccaccio, J. P. Abastado, J. D. Lifson, L. O. Arthur, Y. Rivière, J. M. Heard, and O. Schwartz.** 2001. MHC-I-restricted presentation of HIV-1 virion antigens without viral replication. Nat. Med. **7:**344–349.
12. **Butler, S. L., E. P. Johnson, and F. D. Bushman.** 2002. Human immunodeficiency virus cDNA metabolism: notable stability of two-long terminal repeat circles. J. Virol. **76:**3739–3747.
13. **Cameron, P., M. Pope, A. Granelli-Piperno, and R. M. Steinman.** 1996. Dendritic cells and the replication of HIV-1. J. Leukoc. Biol. **59:**158–171.
14. **Canque, B., Y. Bakri, S. Camus, M. Yagello, A. Benjouad, and J. C. Gluckman.** 1999. The susceptibility to X4 and R5 human immunodeficiency virus-1 strains of dendritic cells derived in vitro from $CD34^+$ hematopoietic progenitor cells is primarily determined by their maturation stage. Blood **93:**3866–3875.
15. **Connor, R. I., B. K. Chen, S. Choe, and N. R. Landau.** 1995. Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. Virology **206:**935–944.
16. **Drose, S., and K. Altendorf.** 1997. Bafilomycins and concanamycins as inhibitors of V-ATPases and P-ATPases. J. Exp. Biol. **200:**1–8.
17. **Feinberg, H., D. A. Mitchell, K. Drickamer, and W. I. Weis.** 2001. Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. Science **294:**2163–2166.
18. **Frank, I., M. Piatak, Jr., H. Stoessel, N. Romani, D. Bonnyay, J. D. Lifson, and M. Pope.** 2002. Infectious and whole inactivated simian immunodeficiency viruses interact similarly with primate dendritic cells (DCs): differential intracellular fate of virions in mature and immature DCs. J. Virol. **76:**2936–2951.
19. **Geijtenbeek, T. B., D. S. Kwon, R. Torensma, S. J. Van Vliet, G. C. Van Duijnhoven, J. Middel, I. L. Cornelissen, H. Nottet, V. KewalRamani, D. Littman, C. G. Figdor, and Y. Van Kooyk.** 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell **100:**587–597.
20. **Goxe, B., N. Latour, J. Bartholeyns, J. L. Romet-Lemonne, and M. Chokri.** 1998. Monocyte-derived dendritic cells: development of a cellular processor for clinical applications. Res. Immunol. **149:**643–646.
21. **Granelli-Piperno, A., E. Delgado, V. Finkel, W. Paxton, and R. M. Steinman.** 1998. Immature dendritic cells selectively replicate macrophagetropic (M-tropic) human immunodeficiency virus type 1, while mature cells efficiency transmit both M- and T-tropic virus to T cells. J. Virol. **72:**2733–2737.
22. **Granelli-Piperno, A., V. Finkel, E. Delgado, and R. M. Steinman.** 1999. Virus replication begins in dendritic cells during the transmission of HIV-1 from mature dendritic cells to T cells. Curr. Biol. **9:**21–29.
23. **Granelli-Piperno, A., A. Golebiowska, C. Trumpfheller, F. P. Siegal, and R. M. Steinman.** 2004. HIV-1-infected monocyte-derived dendritic cells do not undergo maturation but can elicit IL-10 production and T cell regulation. Proc. Natl. Acad. Sci. USA **101:**7669–7674.
24. **Granelli-Piperno, A., B. Moser, M. Pope, D. Chen, Y. Wei, F. Isdell, U. O'Doherty, W. Paxton, R. Koup, S. Mojsov, N. Bhardwaj, I. Clark-Lewis, M. Baggiolini, and R. M. Steinman.** 1996. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors. J. Exp. Med. **184:**2433–2438.
25. **Greenhead, P., P. Hayes, P. S. Watts, K. G. Laing, G. E. Griffin, and R. J. Shattock.** 2000. Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. J. Virol. **74:**5577–5586.
26. **Gummuluru, S., V. N. KewalRamani, and M. Emerman.** 2002. Dendritic cell-mediated viral transfer to T cells is required for human immunodeficiency virus type 1 persistence in the face of rapid cell turnover. J. Virol. **76:**10692–10701.
27. **Gummuluru, S., M. Rogel, L. Stamatatos, and M. Emerman.** 2003. Binding of human immunodeficiency virus type 1 to immature dendritic cells can occur independently of DC-SIGN and mannose binding C-type lectin receptors via a cholesterol-dependent pathway. J. Virol. **77:**12865–12874.
28. **Hu, Q., I. Frank, V. Williams, J. J. Santos, P. Watts, G. E. Griffin, J. P. Moore, M. Pope, and R. J. Shattock.** 2004. Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. J. Exp. Med. **199:**1065–1075.
29. **Igakura, T., J. C. Stinchcombe, P. K. Goon, G. P. Taylor, J. N. Weber, G. M. Griffiths, Y. Tanaka, M. Osame, and C. R. Bangham.** 2003. Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. Science **299:**1713–1716.
30. **Jameson, B., F. Baribaud, S. Pohlmann, D. Ghavimi, F. Mortari, R. W. Doms, and A. Iwasaki.** 2002. Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in humans and rhesus macaques. J. Virol. **76:**1866–1875.
31. **Jolly, C., K. Kashefi, M. Hollinshead, and Q. J. Sattentau.** 2004. HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. J. Exp. Med. **199:**283–293.
32. **Kawamura, T., S. S. Cohen, D. L. Borris, E. A. Aquilino, S. Glushakova, L. B. Margolis, J. M. Orenstein, R. E. Offord, A. R. Neurath, and A. Blauvelt.** 2000. Candidate microbicides block HIV-1 infection of human immature Langerhans cells within epithelial tissue explants. J. Exp. Med. **192:**1491–1500.
33. **Kawamura, T., M. Qualbani, E. K. Thomas, J. M. Orenstein, and A. Blauvelt.** 2001. Low levels of productive HIV infection in Langerhans cell-like dendritic cells differentiated in the presence of TGF-β1 and increased viral replication with CD40 ligand-induced maturation. Eur. J. Immunol. **31:**360–368.
34. **Kilby, J. M., S. Hopkins, T. M. Venetta, B. DiMassimo, G. A. Cloud, J. Y. Lee, L. Alldredge, E. Hunter, D. Lambert, D. Bolognesi, T. Matthews, M. R. Johnson, M. A. Nowak, G. M. Shaw, and M. S. Saag.** 1998. Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. Nat. Med. **4:**1302–1307.
35. **Klagge, I. M., and S. Schneider-Schaulies.** 1999. Virus interactions with dendritic cells. J. Gen. Virol. **80:**823–833.
36. **Kwon, D. S., G. Gregorio, N. Bitton, W. A. Hendrickson, and D. R. Littman.** 2002. DC-SIGN-mediated internalization of HIV is required for trans-enhancement of T cell infection. Immunity **16:**135–144.
37. **Le Gall, S., L. Erdtmann, S. Benichou, C. Berlioz-Torrent, L. X. Liu, R. Benarous, J. M. Heard, and O. Schwartz.** 1998. Nef interacts with μ subunits of clathrin adaptor complexes and reveals a cryptic sorting signal in MHC-I molecules. Immunity **8:**483–495.
38. **Lin, C. L., A. K. Sewell, G. F. Gao, K. T. Whelan, R. E. Phillips, and J. M. Austyn.** 2000. Macrophage-tropic HIV induces and exploits dendritic cell chemotaxis. J. Exp. Med. **192:**587–594.
39. **Liu, R., W. A. Paxton, S. Choe, D. Ceradini, S. R. Martin, R. Horuk, M. E. MacDonald, H. Stuhlmann, R. A. Koup, and N. R. Landau.** 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell **86:**367–377.
40. **Maréchal, V., F. Clavel, J. M. Heard, and O. Schwartz.** 1998. Cytosolic Gag p24 as an index of productive entry of human immunodeficiency virus type 1. J. Virol. **72:**2208–2212.
41. **McDonald, D., L. Wu, S. M. Bohks, V. N. KewalRamani, D. Unutmaz, and T. J. Hope.** 2003. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. Science **300:**1295–1297.
42. **Messmer, D., R. Ignatius, C. Santisteban, R. M. Steinman, and M. Pope.** 2000. The decreased replicative capacity of simian immunodeficiency virus SIVmac239Δ*nef* is manifest in cultures of immature dendritic cells and T cells. J. Virol. **74:**2406–2413.
43. **Moris, A., C. Nobile, F. Buseyne, F. Porrot, J. P. Abastado, and O. Schwartz.** 2004. DC-SIGN promotes exogenous MHC-I-restricted HIV-1 antigen presentation. Blood **103:**2648–2654.
44. **Nobile, C., A. Moris, F. Porrot, N. Sol-Foulon, and O. Schwartz.** 2003. Inhibition of human immunodeficiency virus type 1 Env-mediated fusion by DC-SIGN. J. Virol. **77:**5313–5323.
45. **Petit, C., F. Buseyne, C. Boccaccio, J. P. Abastado, J. M. Heard, and O. Schwartz.** 2001. Nef is required for efficient HIV-1 replication in cocultures of dendritic cells and lymphocytes. Virology **286:**225–236.
46. **Pope, M., M. G. Betjes, N. Romani, H. Hirmand, P. U. Cameron, L. Hoffman, S. Gezelter, G. Schuler, and R. M. Steinman.** 1994. Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. Cell **78:**389–398.
47. **Pope, M., S. Gezelter, N. Gallo, L. Hoffman, and R. M. Steinman.** 1995. Low levels of HIV-1 infection in cutaneous dendritic cells promote extensive viral

replication upon binding to memory CD4+ T cells. J. Exp. Med. **182:**2045–2056.

48. **Pope, M., and A. T. Haase.** 2003. Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. Nat Med. **9:**847–852.
49. **Reece, J. C., A. J. Handley, E. J. Anstee, W. A. Morrison, S. M. Crowe, and P. U. Cameron.** 1998. HIV-1 selection by epidermal dendritic cells during transmission across human skin. J. Exp Med. **187:**1623–1631.
50. **Sanders, R. W., E. C. de Jong, C. E. Baldwin, J. H. Schuitemaker, M. L. Kapsenberg, and B. Berkhout.** 2002. Differential transmission of human immunodeficiency virus type 1 by distinct subsets of effector dendritic cells. J. Virol. **76:**7812–7821.
51. **Schwartz, O., J. L. Virelizier, L. Montagnier, and U. Hazan.** 1990. A microtransfection method using luciferase gene for the study of human immunodeficiency virus long terminal repeat activity. Gene **88:**197–205.
52. **Trono, D.** 1992. Partial reverse transcripts in virions from human immunodeficiency and murine leukemia viruses. J. Virol. **66:**4893–4900.
53. **Trouplin, V., F. Salvatori, F. Cappello, V. Obry, A. Brelot, N. Heveker, M. Alizon, G. Scarlatti, F. Clavel, and F. Mammano.** 2001. Determination of coreceptor usage of human immunodeficiency virus type 1 from patient plasma samples by using a recombinant phenotypic assay. J. Virol. **75:**251–259.
54. **Trumpfheller, C., C. G. Park, J. Finke, R. M. Steinman, and A. Granelli-Piperno.** 2003. Cell type-dependent retention and transmission of HIV-1 by DC-SIGN. Int. Immunol. **15:**289–298.
55. **Turville, S. G., J. Arthos, K. M. Donald, G. Lynch, H. Naif, G. Clark, D. Hart, and A. L. Cunningham.** 2001. HIV gp120 receptors on human dendritic cells. Blood **98:**2482–2488.
56. **Turville, S. G., P. U. Cameron, A. Handley, G. Lin, S. Pöhlmann, R. W. Doms, and A. L. Cunningham.** 2002. Diversity of receptors binding HIV on dendritic cell subsets. Nat. Immunol. **3:**975–983.
57. **Turville, S. G., J. J. Santos, I. Frank, P. U. Cameron, J. Wilkinson, M. Miranda-Saksena, J. Dable, H. Stossel, N. Romani, M. Piatak, Jr., J. D. Lifson, M. Pope, and A. L. Cunningham.** 2004. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. Blood **103:**2170–2179.
58. **Wu, L., T. D. Martin, M. Carrington, and V. N. KewalRamani.** 2004. Raji B cells, misidentified as THP-1 cells, stimulate DC-SIGN-mediated HIV transmission. Virology **318:**17–23.
59. **Wu, L., T. D. Martin, Y. C. Han, S. K. Breun, and V. N. KewalRamani.** 2004. Trans-dominant cellular inhibition of DC-SIGN-mediated HIV-1 transmission. Retrovirology **1:**14.
60. **Zaitseva, M., A. Blauvelt, S. Lee, C. K. Lapham, V. Klaus-Kovtun, H. Mostowski, J. Manischewitz, and H. Golding.** 1997. Expression and function of CCR5 and CXCR4 on human Langerhans cells and macrophages: implications for HIV primary infection. Nat. Med. **3:**1369–1375.
61. **Zhang, H., G. Dornadula, and R. J. Pomerantz.** 1996. Endogenous reverse transcription of human immunodeficiency virus type 1 in physiological microenviroments: an important stage for viral infection of nondividing cells. J. Virol. **70:**2809–2824.
62. **Zhu, T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup, and D. D. Ho.** 1993. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science **261:**1179–1181.
63. **Zoeteweij, J. P., H. Golding, H. Mostowski, and A. Blauvelt.** 1998. Cytokines regulate expression and function of the HIV coreceptor CXCR4 on human mature dendritic cells. J. Immunol. **161:**3219–3223.